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Plasma variations of biomarkers for muscle damage in male nondisabled and spinal cord injured subjects

Sandra Loerakker, MSc;[1*] Elise S. Huisman, MSc;[1] Henk A. M. Seelen, PhD;[2] Jan F. C. Glatz, PhD;[3] Frank P. T. Baaijens, PhD;[1] Cees W. J. Oomens, PhD;[1] Dan L. Bader, PhD[1,4]
[1]Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands; [2]Department of Research, Adelante Centre of Expertise in Rehabilitation and Audiology, Hoensbroek, the Netherlands; [3]Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, the Netherlands; [4]Faculty of Health Sciences, University of Southampton, Southampton, United Kingdom

Abstract—Deep pressure ulcers represent a major problem for individuals with spinal cord injury (SCI), with the initial damage often hidden underneath intact skin. Accordingly, early detection is difficult and treatment is problematic. In the present study, circulatory levels of biomarkers for muscle damage were investigated to explore their potential in the early detection of deep pressure ulcers. Baseline concentrations of creatine kinase, myoglobin (Mb), heart-type fatty acid binding protein (H-FABP), and C-reactive protein (CRP) were measured in small groups of nondisabled (age 39–66 yr) subjects and subjects with SCI (age 40–68 yr, American Spinal Injury Association grade A–B, level of injury thoracic 11 to lumbar 3) over a period of 5 days. Each subject exhibited a unique concentration profile for all markers, although some correlations were observed; for example, Mb and H-FABP were correlated for both subject groups. No significant differences were found in marker concentrations between the two subject groups, although a trend toward higher CRP levels was observed in the SCI subjects. Furthermore, one SCI subject with a category II pressure ulcer exhibited higher H-FABP and CRP concentrations than all other subjects. Because the variations in each of the marker concentrations were smaller than the predicted increases after pressure ulcers, this combination of plasma markers may prove appropriate for the early detection of deep pressure ulcers.

Key words: C-reactive protein, creatine kinase, deep tissue injury, early detection, heart-type fatty acid binding protein, muscle damage, myoglobin, plasma concentrations, pressure ulcers, spinal cord injury.

INTRODUCTION

A pressure ulcer is a local lesion to the skin and/or underlying tissues resulting from prolonged mechanical loading involving pressure alone or in combination with shear and/or friction [1]. Two types of pressure ulcers can be distinguished. Superficial pressure ulcers involve the skin layer only, while deep pressure ulcers are often initiated in deep muscle tissue adjacent to bony prominences [2]. The latter ulcers only become apparent at the skin surface when tissue destruction is extensive, having progressed up from the muscle layers. Accordingly, early detection and prevention is problematic and subsequent treatment is prolonged, with a variable prognosis.

Abbreviations: ASIA = American Spinal Injury Association, CK = creatine kinase, CRP = C-reactive protein, ELISA = enzyme-linked immunosorbent assay, H-FABP = heart-type fatty acid binding protein, Mb = myoglobin, SCI = spinal cord injury, SCI-A = SCI active, SCI-NA = SCI nonactive, SCI+PU = SCI plus pressure ulcer, T = thoracic, U/L = units per liter.

[*]**Address all correspondence to Sandra Loerakker, PhD; Department of Biomedical Engineering, Eindhoven University of Technology, PO Box 513, 5600 MB Eindhoven, the Netherlands; +31(0)40-247-4830; fax: +31(0)40-247-3027. Email: s.loerakker@tue.nl**

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Subjects with spinal cord injury (SCI) are particularly susceptible to deep pressure ulcers because of their inherent impaired sensitivity, disuse muscle atrophy, and impaired vascularity [3–4]. Accordingly, the prevalence of pressure ulcers in this population has been studied frequently to identify risk factors and determine individual susceptibility to pressure ulcer development [5–6]. For example, Rodriguez and Claus-Walker postulated that the established change in skin resistance to external forces post-SCI could be attributed to breakdown of the structural protein collagen [7]. They reported increased levels of the collagen degradation products hydroxylysine and hydroxyproline in a small group of SCI subjects, suggesting decreased skin stiffness and strength. Other research for identifying persons at risk for pressure ulcers examined the metabolites of sweat collected at the skin surface. In compressed skin, the impairment of both blood supply and lymphatic drainage could lead to increased production of urea, lactate, and urate [8–9]. However, the simple method of sweat collection requires a significant sweat volume for analysis and thus may not prove appropriate for SCI subjects with altered or reduced sudomotor function.

In the present study, circulatory levels of biomarkers for muscle damage were investigated to explore the possibility of using them for detection of deep pressure ulcers. Three markers of muscle damage and one marker of inflammation were chosen for investigation:

- Creatine kinase (CK), an enzyme with a combined molecular mass of 86 kDa involved in cellular energetics and muscle metabolism, which is mostly found in skeletal and cardiac muscle and the brain [10].
- Myoglobin (Mb), a cytoplasmic hemoprotein of 18 kDa, which is present in skeletal and cardiac muscle [10].
- Heart-type fatty acid binding protein (H-FABP), a protein of 15 kDa involved in the cellular uptake, transport, and metabolism of fatty acids in skeletal and cardiac muscle and the kidneys [10].
- C-reactive protein (CRP), a ubiquitous marker in inflammation, tissue damage, and infection [11]. CRP levels are generally higher in SCI subjects compared with nondisabled controls because of their chronic inflammatory state [12].

The concentrations of these proteins in the circulation are determined by their release from the injured tissue and the clearance by the kidneys [13]. In a study with nondisabled human volunteers, levels of CK, Mb, and H-FABP all increased after eccentric exercise [14]. The time to reach peak concentration and subsequently return to baseline levels was shorter for Mb and H-FABP than CK, which can be explained by the differences in molecular mass of these proteins. These markers have also been used to detect cardiac muscle damage. Skeletal muscle damage can be distinguished from cardiac muscle damage by calculating the ratio of the Mb concentration over the H-FABP concentration, which is considerably higher in the case of skeletal muscle damage (with ratios of 20–70) when compared with cardiac muscle damage (with ratios of ~5) [15]. With respect to pressure ulcers, several studies have reported considerable increases in CK levels in serum and wound exudate in animal studies on deep pressure ulcers [16–18]. Furthermore, higher serum levels of CRP were observed in SCI subjects with pressure ulcers than in subjects without ulcers [12,19–20].

This study represents a preliminary investigation of the basal circulatory levels and variations of these four markers in nondisabled subjects and SCI subjects with a low-level lesion (injury level thoracic [T] 11 or lower), in which one SCI subject had a pressure ulcer. To assess whether these markers are appropriate for early detection, we asked the following questions:

1. What are the baseline circulatory levels of these markers in SCI subjects and is there a difference compared with nondisabled individuals?
2. How large are the variations of the markers within a day and during a week?
3. Are the variations smaller than variations expected when a pressure ulcer develops or exists?
4. Are there any relationships between the circulatory levels of these markers?

METHODS

Participants

The participants in this study included eight male subjects with traumatic SCI (mean age 56, range 40–68) who were former patients of a rehabilitation center (Adelante; Hoensbroek, the Netherlands). The participant information is detailed in **Table 1**. One subject, 11 yr postinjury, presented with a category II pressure ulcer at the sacrum (ulcer had been present for a year) and had American Spinal Injury Association (ASIA) Impairment Scale classification A [21], with neurologic level of injury T12 (SCI plus pressure ulcer [SCI+PU] group, $n = 1$). This subject also suffered from diabetes, hypertension, high cholesterol, and had a stoma. Of the remaining participants (SCI group,

Table 1.
Characteristics of study participants.

Characteristic	Control (n = 7)	SCI (n = 7)	SCI+PU (n = 1)
Age (years)			
Median	55	56	59
Range	39–66	40–68	—
BMI (kg/m^2)			
Median	24	25	31
Range	21–29	22–28	—
ASIA Grade			
A	—	6	1
B	—	1	—
Injury Level			
T11	—	2	—
T12	—	—	1
L1	—	1	—
L2	—	2	—
L3	—	2	—
Years Postinjury			
Median	—	15	11
Range	—	1–33	—
Previous Pressure Ulcers			
Median	—	1	3
Range	—	0–3	—
Comorbidities			
Diabetes	—	1	1
Hypertension	—	2	1
High Cholesterol	—	1	1

ASIA = American Spinal Injury Association, BMI = body mass index, L = lumbar, SCI = spinal cord injury, SCI+PU = SCI plus pressure ulcer, T = thoracic.

n = 7), who were between 1 and 33 yr postinjury, six had an ASIA-A classification and one had an ASIA-B classification [21]. The neurological level of injury in these seven subjects was between T11 and lumbar 3. One of these subjects had diabetes and high cholesterol and two had hypertension. To investigate the effect of exercise on the marker levels, we divided the SCI group into two subgroups, SCI active (SCI-A) and SCI nonactive (SCI-NA), based on the individual motor function of the subjects. SCI-A subjects regularly walked with crutches or a parapodium during the week, while SCI-NA subjects did not. For comparison, a control group of age-matched male subjects was recruited from volunteers at the Eindhoven University of Technology, consisting of seven nondisabled participants without any known comorbidities (control group, n = 7; **Table 1**).

Experimental Protocol

Blood was drawn by a standard venipuncture from all subjects on 5 consecutive days. One blood sample per day was taken in the early afternoon after lunch for 4 days. On the fifth day, three samples were taken: in the morning (after breakfast), early afternoon, and late afternoon. Blood samples were collected in two 5 mL Vacutainer collection tubes (BD; Franklin Lakes, New Jersey), which contained Olefin gel, heparin, and lithium to prevent coagulation. Blood was centrifuged at 3,000 rpm for 10 min. Plasma was transferred into two vials, one of which was analyzed for CK, Mb, and CRP content. The vial used for H-FABP analysis was stored at –80 °C and subsequently tested with an enzyme-linked immunosorbent assay (ELISA). Basic clinical and demographic information was obtained through a questionnaire. Furthermore, all participants filled in a diary to monitor their level of exercise related to work, sports, or any special circumstances.

Biochemical Analysis

The CK, Mb, and CRP contents were determined using a photometer (Analyzer Roche type Modular; Roche, Switzerland). CK catalyzes the reaction of phosphocreatine with adenosine diphosphate to creatine and adenosine triphosphate. The content of the reduced form of nicotinamide adenine dinucleotide phosphate, which is directly proportional to the CK content, was measured photometrically. The detection range was 3 to 2,300 units per liter (U/L). The CRP content was also determined by photometry, with a detection range of 1 to 350 mg/L. Electrochemiluminescence, a sandwich-principle–based immunoassay, was used for Mb detection in plasma samples, with a detection range of 21 to 3,000 g/L. The coefficients of variation for total precision of the CK, CRP, and Mb concentrations were <1.4, <6.2, and <5.2 percent, respectively. H-FABP ELISAs (Hycult Biotech; Uden, the Netherlands) were used to detect the concentration of H-FABP in all samples. This assay is a solid-phase ELISA based on the sandwich principle. Samples and standard were tested in duplicate and averaged. The detection range of the ELISA was 0.1 to 25.0 ng/mL.

Statistical Analysis

To compare the marker concentrations of the nondisabled and SCI subjects, the median marker concentrations over the 5 days were calculated for each individual. Subsequently, the presence of significant differences in median marker levels between the groups was determined

using the nonparametric Wilcoxon rank sum test. The presence of diurnal variations in marker levels was investigated by applying the nonparametric Friedman test to the marker concentrations that were measured in the morning and the early and late afternoon of the same day, followed by a Bonferroni post hoc test. Correlations between the marker levels were assessed using the Spearman rank correlation coefficient. Test results were considered significant if $p < 0.05$.

RESULTS

Intersubject Variations

The marker concentrations of the four proteins on the 5 consecutive days are shown in **Figure 1**. These concentrations indicate that the majority of intrasubject variations were small compared with the intersubject variations in marker levels, even within groups. For CK in the control group, the largest values for both concentration and variation were observed in subject 3 (185–599 U/L). In the SCI group without pressure ulcers, the greatest concentrations were present in subject 7 (306–467 U/L). The SCI+PU subject showed relatively low CK levels with a small variation (30–70 U/L). For Mb, only minor intersubject variations were observed for the control group, with median concentrations ranging from 43 to 67 ng/mL. The greatest Mb concentrations were found in subject 4 (128–171 ng/mL) of the SCI group. The Mb levels of the SCI+PU subject were comparable with both other subject groups. For H-FABP, the intersubject variations were also greater than the intrasubject variations, although the differences in the former were smaller than for CK. SCI subject 7 (1.6–7.1 ng/mL) showed the largest variation in H-FABP levels, and the highest concentrations were observed for the SCI+PU subject (6.7–9.3 ng/mL). The largest variations in CRP levels were found in control subject 6 (2–7 g/mL), and SCI subjects 3 (8–14 g/mL), 4 (12–22 g/mL), and 5 (4–14 g/mL). The highest CRP concentrations were present in the SCI+PU subject (24–29 g/mL).

Diurnal Variations

Figure 2 shows the marker concentrations of the samples that were taken in the morning and the early and late afternoon of the same day. Close examination of the diurnal data using the Friedman test indicated some significant differences between time points in the group of SCI subjects. In particular, the concentrations of Mb in the morning were greater than those measured in the late afternoon ($p = 0.03$). For H-FABP, the morning concentrations were greater than those in the early afternoon ($p = 0.02$). For the other markers, no significant differences in concentration were present between the different time points in both the control and SCI groups.

Comparison of Groups

The group data of the marker concentrations on the 5 consecutive days are summarized in **Table 2**. For the group comparisons, the SCI group was presented both as a single group and divided into two groups according to individual motor function; SCI active (SCI-A, subjects 1–3, 7) and SCI nonactive (SCI-NA, subjects 4–6). The group data are also displayed in **Figure 3**. The Wilcoxon rank sum test revealed no significant differences between the groups of nondisabled and SCI subjects for any of the markers. Also, no significant differences were found between the SCI-A and SCI-NA groups.

The SCI+PU subject showed relatively low CK concentrations compared with the other groups (**Figure 3(a)**). Furthermore, the CK concentrations in the SCI-A subjects were larger than the concentrations in the SCI-NA group, although this result could not be confirmed by statistical analysis ($p = 0.06$). The median Mb concentrations were comparable in all groups (**Figure 3(b)**). For H-FABP, similar concentrations were present in nondisabled and SCI subject groups (**Figure 3(c)**). The median ratio of Mb over H-FABP showed values within the range for skeletal muscle damage (20–70 [15]) for the control group and the SCI groups without pressure ulcers (**Table 2**). The SCI+PU subject exhibited relatively high H-FABP concentrations, with a correspondingly reduced value of the Mb/H-FABP ratio. However, similar values of this ratio were also observed for some individuals within the SCI group. In general, the CRP levels were higher in the SCI subjects than in the control group, although this result could not be confirmed by statistical analysis ($p = 0.14$). Furthermore, we observed a trend toward higher CRP concentrations in the SCI-NA subjects compared with the SCI-A subjects ($p = 0.23$) (**Figure 3(d)**).

Correlations Between Marker Concentrations

The Spearman rank correlation coefficient revealed significant correlations in four pairs of markers in the SCI group and three in the group of nondisabled volunteers (**Table 3**). Positive correlations between CK and H-FABP and Mb and H-FABP were present in both groups. In

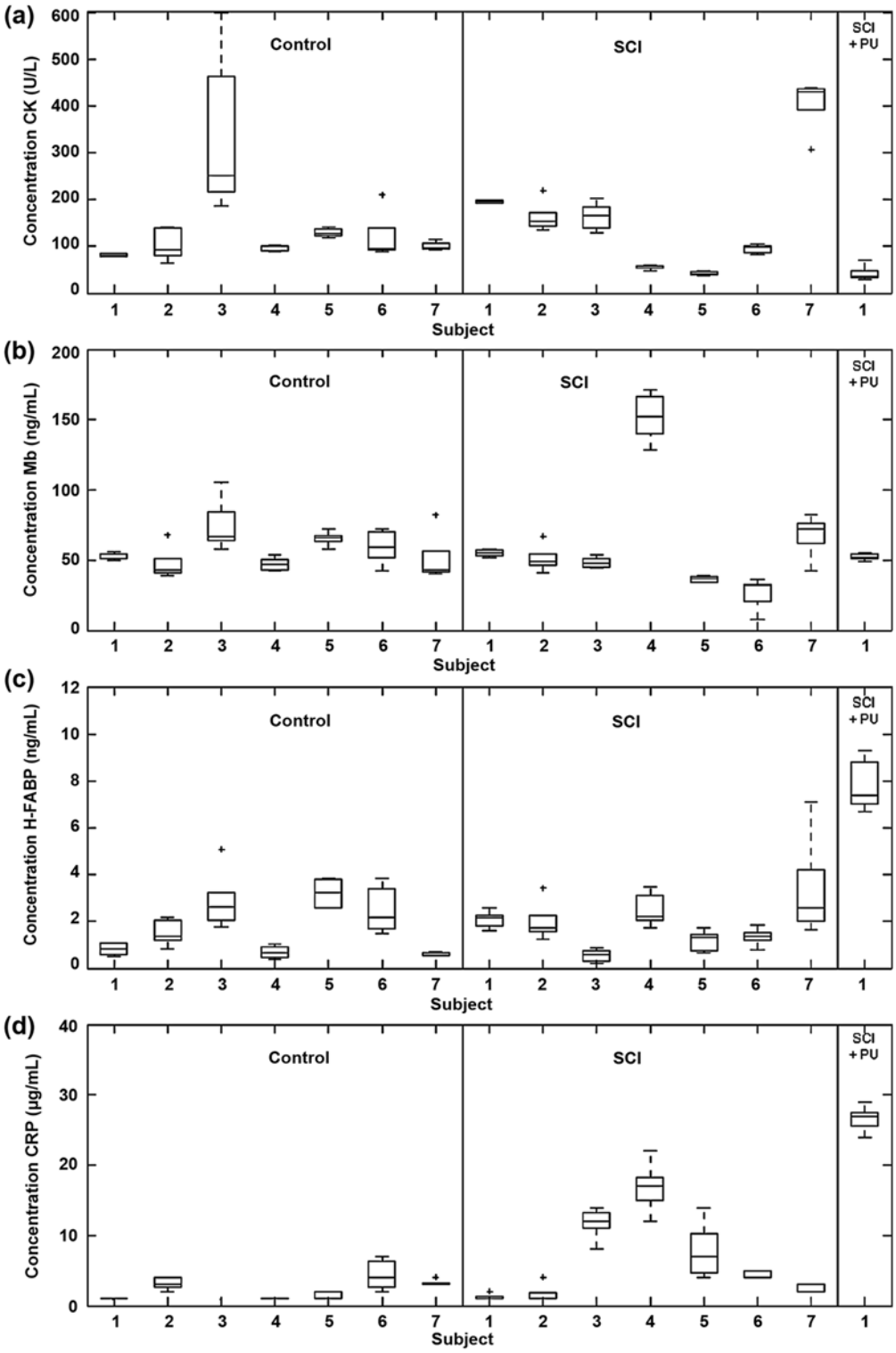

Figure 1.
Concentrations of **(a)** creatine kinase (CK), **(b)** myoglobin (Mb), **(c)** heart-type fatty acid binding protein (H-FABP), and **(d)** C-reactive protein (CRP) for all subjects in samples taken on 5 consecutive days. SCI = spinal cord injury, SCI+PU = SCI plus pressure ulcer, U/L = units per liter.

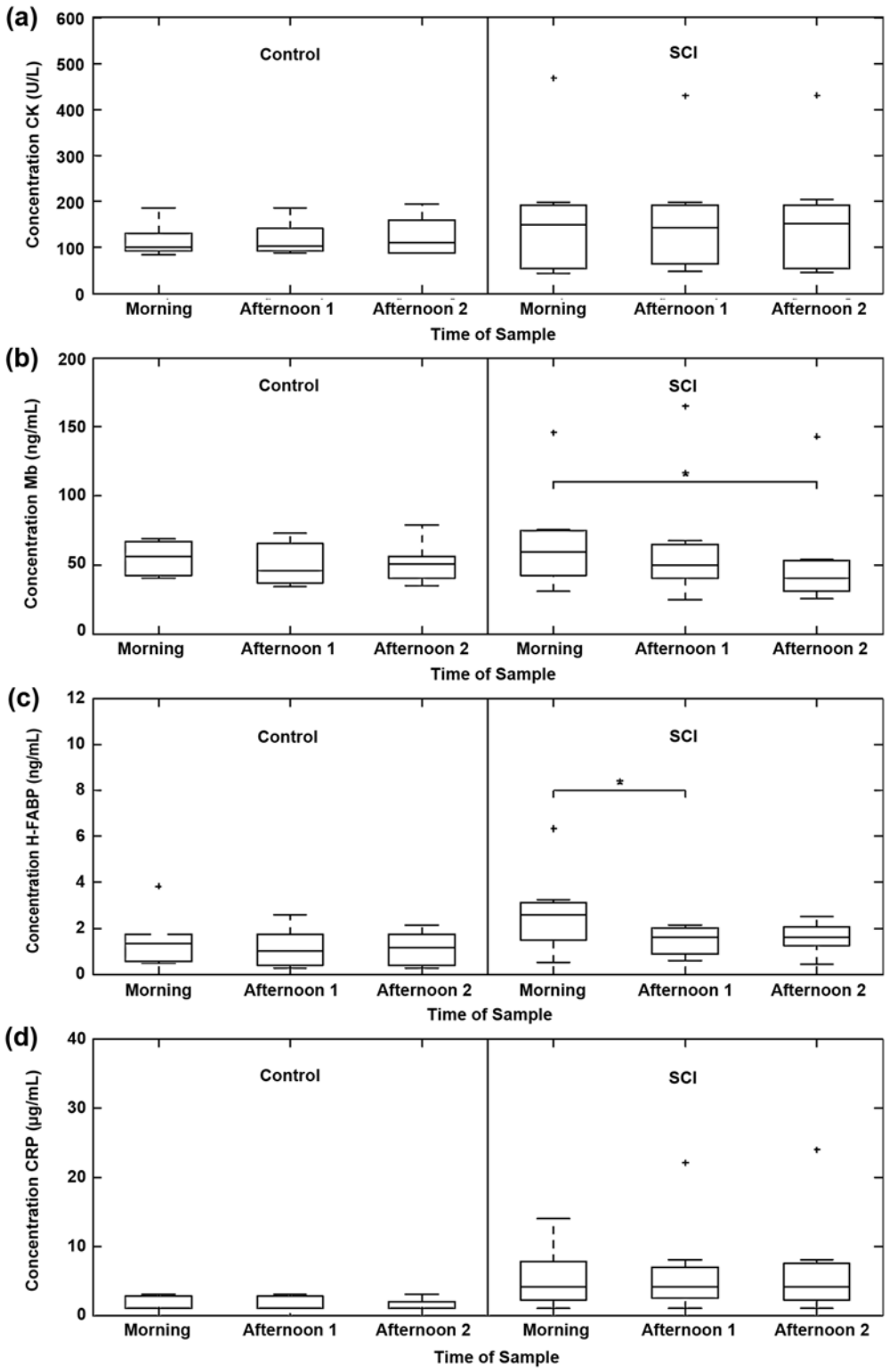

Figure 2.
Concentrations of **(a)** creatine kinase (CK), **(b)** myoglobin (Mb), **(c)** heart-type fatty acid binding protein (H-FABP), and **(d)** C-reactive protein (CRP) for all subjects in 3 samples taken in morning, and early (1) and late (2) afternoon of same day. *Significant difference between time points. SCI = spinal cord injury, U/L = units per liter.

addition, there was a positive correlation between CK and Mb in the control group. By contrast, there were negative correlations between CK and CRP and between H-FABP and CRP in the SCI group.

DISCUSSION

The present study was conducted to investigate the basal circulatory levels and variations of three markers of muscle damage and one marker of inflammation in control and SCI subjects with lesions in the lower T or lumbar spines. No significant differences were found between the groups of nondisabled and SCI subjects for any markers. The CRP levels in the SCI subjects were generally higher than in the nondisabled controls, although this result could not be confirmed by statistical analysis ($p = 0.14$). The one SCI+PU subject revealed relatively high H-FABP and CRP values compared with all other subjects. In addition, close examination of the SCI group revealed trends toward higher CK ($p = 0.06$) and lower CRP ($p = 0.23$) baseline values in those SCI subjects with motor function (SCI-A) compared with those lacking motor function (SCI-NA).

Table 2.
Concentrations of markers of muscle damage (CK, Mb, and H-FABP), and inflammation (CRP) in control, SCI, and SCI+PU groups on 5 consecutive days. SCI group was presented both as single group and divided into two groups according to individual motor function; SCI active (SCI-A, subjects 1–3, 7) and SCI nonactive (SCI-NA, subjects 4–6).

Group	CK (U/L)	Mb (ng/mL)	H-FABP (ng/mL)	Ratio (Mb/H-FABP)	CRP (µg/mL)
Control ($n = 7$)					
Median	102	54	1.3	39	1
Range	64–599	34–105	0.3–5.1	15–126	1–7
SCI ($n = 7$)					
Median	144	50	1.7	29	4
Range	38–467	8–171	0.2–7.1	6–200	1–24
SCI-A ($n = 4$)					
Median	194	53	1.8	29	2
Range	130–467	29–82	0.2–7.1	10–200	1–14
SCI-NA ($n = 3$)					
Median	54	37	1.5	29	5
Range	38–103	8–171	0.7–3.5	6–88	3–24
SCI+PU ($n = 1$)					
Median	33	54	8.6	6	27
Range	27–70	49–55	6.7–9.3	6–8	24–30

CK = creatine kinase, CRP = C-reactive protein, H-FABP = heart-type fatty acid binding protein, Mb = myoglobin, SCI = spinal cord injury, SCI-A = SCI active, SCI-NA = SCI nonactive, SCI+PU = SCI plus pressure ulcer, U/L = units per liter.

The results of this study reveal a small intrasubject range for all markers when compared with the intersubject differences. Each subject has a different range of marker values, which could, in part, be explained by their different levels of activity as reported in the individual diaries. The present findings reveal significant diurnal variations within the SCI subjects for Mb and H-FABP, with morning values generally elevated compared with those collected later in the day, a trend previously reported with nondisabled subjects [22–23]. Although daily activities might have been predicted to increase marker values, the observed decrease may be a direct result of an increased glomerular filtration rate during the day [23].

The SCI group demonstrated relatively high CRP levels compared with the control group, supporting findings from recent studies [12,24]. CRP plasma levels are affected in multiple situations, increasing rapidly after both tissue damage and many forms of inflammation and infection. This marker has often been used with SCI subjects, and its increase has also been reported in individuals with pressure ulcers [12,19–20]. However, other proinflammatory cytokines, such as interleukin-2 receptor and interleukin-6, may also be appropriate, as has recently been reported in studies with SCI subjects [25–26].

Close examination of the SCI group revealed trends toward higher baseline concentrations of CK for the SCI-A subjects compared with both the SCI-NA and control groups. The SCI-A group regularly walked with crutches or a parapodium during the week, which represents intensive and strenuous exercise for the muscles, thereby releasing relatively large amounts of CK into the circulation. This result matches the findings that nondisabled subjects who regularly exercise tend to have higher circulatory levels of CK than their sedentary counterparts [27–28]. In addition, relatively low CRP levels were present in the SCI-A group compared with the SCI-NA group, which is consistent with the findings of a recent study in which decreasing levels of CRP were reported with increasing levels of activity in a chronic SCI group [20].

Correlational analyses yielded positive relationships that were statistically significant between CK and H-FABP and Mb and H-FABP for both SCI and control groups, and a positive correlation between CK and Mb was also found in the control group (**Table 3**). These findings imply a

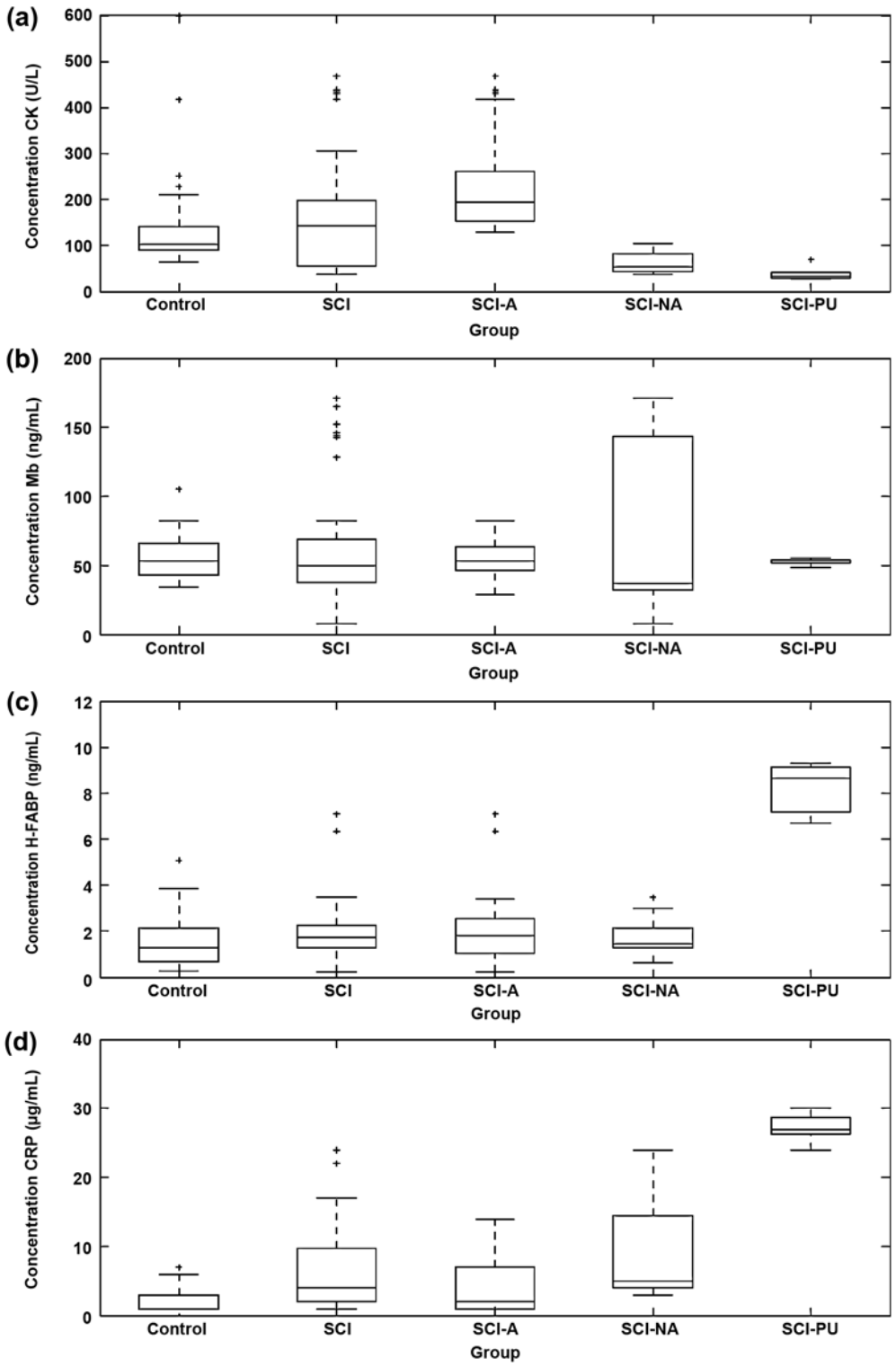

Figure 3.
Group results of marker concentrations of **(a)** creatine kinase (CK), **(b)** myoglobin (Mb), **(c)** heart-type fatty acid binding protein (H-FABP), and **(d)** C-reactive protein (CRP) on 5 consecutive days. SCI group was presented both as single group and divided into two groups according to motor function. SCI = spinal cord injury, SCI-A = SCI active, SCI-NA = SCI nonactive, SCI-PU = SCI plus pressure ulcer, U/L = units per liter.

Table 3.
Significant Spearman rank correlation coefficients between combinations of markers for both control (lower left part of table) and SCI (upper right part of table) group. NS indicates correlation coefficient was not significant.

Control	SCI			
	CK	**Mb**	**H-FABP**	**CRP**
CK		NS	0.28	−0.61
Mb	0.59		0.68	NS
H-FABP	0.60	0.74		−0.29
CRP	NS	NS	NS	

CK = creatine kinase, CRP = C-reactive protein, H-FABP = heart-type fatty acid binding protein, Mb = myoglobin, NS = not significant, SCI = spinal cord injury.

simultaneous increase in Mb, H-FABP, and CK, the former two markers being of similar molecular size (15–18 kDa). By contrast, the larger CK molecule (86 kDa) would lead to a predicted delay in its increase compared with H-FABP and Mb. Indeed, research examining myocardial infarction and exercise revealed an increase in Mb and H-FABP at similar time points and a delayed offset in CK [14,29–30]. In addition, in the SCI group, a significant negative correlation was found between H-FABP and CRP and CK and CRP (**Table 3**), corresponding to the results of Morse et al. in which CRP levels of SCI subjects were negatively correlated with their activity levels [20].

Interestingly, previous research involving Mb and H-FABP levels reported a larger increase in concentration after myocardial infarction than the variations observed in the present study [15,30]. For example, Glatz et al. reported peak levels of Mb up to 20 times the baseline range after myocardial infarction [30]. Additionally, considerable increases of CK levels in serum and wound exudate were reported in animal studies on deep pressure ulcers. Hagisawa et al. found a clear increase in CK concentration after 6 h mechanical loading (from ~500 U/L at baseline to >1,500 U/L after loading) in a pig model [16]. In Sari et al., a clear increase in CK concentration was observed (from ~30 U/L to ~200 U/L) [17]. Furthermore, increased levels of CRP were present in SCI subjects with pressure ulcers compared with subjects without ulcers [12,19–20]. Since these elevations in marker levels were much larger than the intra- and intersubject variations observed in the present study within a period of 5 days, this combination of four markers may prove appropriate for the early detection of deep pressure ulcers.

In the present study, only one volunteer with a pressure ulcer was recruited. This subject had a category II ulcer, which is defined as partial thickness loss of the dermis [1]. This type of pressure ulcer is limited to the skin layers, as opposed to deep pressure ulcers that involve muscle tissue. Since three of the four markers that were used in the present study reflect the presence of muscle damage, it was unlikely that the category II ulcer in this subject would cause increased levels of muscle damage markers. Nevertheless, the same principle as proposed for the early detection of deep pressure ulcers may also hold for skin-confined ulcers. In this case, other markers could be used that reflect the presence of skin damage, such as the collagen degradation products hydroxylisine and hydroxyproline [7].

The SCI subject with the pressure ulcer showed relatively low CK concentrations compared with both the SCI and control subjects, which can be attributed to a decreased activity level. The CRP levels were increased with respect to all individuals without ulcers, presumably as a result of the inflammatory response associated with the pressure ulcer. The H-FABP concentrations in this subject were also high compared to the SCI group and the nondisabled controls, whereas the Mb levels were comparable to the other subject groups. Therefore, the Mb/H-FABP ratio was very low, although it was still within the range reported by Pelsers et al. [23]. Most probably, the large H-FABP concentrations of this subject are still within the baseline variations. Indeed, it is unlikely that this increase was a direct result of the pressure ulcer, as the category II ulcer associated with this subject does not involve muscle tissue. It would be interesting to investigate the plasma concentrations of these four markers in subjects with a category IV ulcer or deep tissue injury, although the latter type of pressure ulcers is particularly difficult to identify.

The selected muscle damage markers are separately not unique indicators for skeletal muscle damage because they are also present in cardiac tissue. The ratio Mb/H-FABP has been reported to distinguish between damage in these two muscle tissues [15]. In the present study, however, comparison between SCI and control subjects was unremarkable, with both cases yielding considerable variations, as was evident in a study on nondisabled subjects with a wide age range [23]. This large variation in the Mb/H-FABP ratio is probably caused by the fact that the baseline H-FABP concentration is relatively small.

The results of this study raise a number of topics to be addressed if markers are to be used to assess early changes in the integrity of skeletal muscles. For example,

the diurnal variations of Mb and H-FABP suggest the importance of withdrawing blood at a fixed time point. Indeed such a recommendation was made in a recent study measuring the release of muscle damage and inflammatory markers in response to eccentric exercise in young, nondisabled subjects [31]. The time period between sample testing is also important. Assuming that the rise in markers is a rapid process and, in the worst case scenario, the pressure ulcer develops very rapidly, it might therefore be advisable to test marker levels every day, particularly with high-risk subjects. For those more susceptible to slowly developing pressure ulcers, a longer time period between sample withdrawals may prove adequate. Finally, as the intrasubject variations in marker levels were smaller than the intersubject variations, it is important to determine the baseline variations of an individual to define a threshold above which an increase in marker levels may indicate the development of a pressure ulcer.

Only a small number of SCI subjects with a low-level lesion participated in the present study. An extended study might also include subjects with higher level lesions, who generally present extended disuse atrophy leading to decreased muscle volume. In such cases, more adipose tissue is present and other more adipose or skin-related markers, such as hydroxylysine or hydroxyproline [32–33], could be included in the blood analysis. In addition, further studies might include female SCI subjects, in which case the hormonal influence on muscle markers would need to be accommodated [23,34].

CONCLUSIONS

In summary, the present study showed that each subject exhibited a unique concentration profile for all four biomarkers that could, in part, be explained by their level of physical activity. These intrasubject variations appeared to be smaller than the intersubject variations. Furthermore, a number of correlations between the different marker concentrations were found for both subject groups. No differences in baseline concentrations were found in CK, Mb, and H-FABP between the SCI and control group. For CRP, relatively high concentrations were observed in the SCI group when compared with the nondisabled controls, although the relatively small group numbers precluded any confirmation by statistical analysis.

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Author Contributions:

Study concept and design: S. Loerakker, E. S. Huisman, H. A. M. Seelen, J. F. C. Glatz, F. P. T. Baaijens, C. W. J. Oomens, D. L. Bader.

Data collection and analysis: S. Loerakker, E. S. Huisman.

Manuscript preparation: S. Loerakker, E. S. Huisman, D. L. Bader.

Study supervision: H. A. M. Seelen, F. P. T. Baaijens, C. W. J. Oomens, D. L. Bader.

Clinical advisor: H. A. M. Seelen.

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Participant Follow-Up*:* The authors do not plan to notify participants of the publication of this study.

REFERENCES

1. NPUAP and EPUAP. Prevention and treatment of pressure ulcers: Clinical practice guideline. Washington (DC): National Pressure Ulcer Advisory Panel; 2009.
2. Bouten CV, Oomens CW, Baaijens FP, Bader DL. The etiology of pressure ulcers: Skin deep or muscle bound? Arch Phys Med Rehabil. 2003;84(4):616–19. [PMID:12690603] http://dx.doi.org/10.1053/apmr.2003.50038
3. Scelsi R. Skeletal muscle pathology after spinal cord injury: Our 20 year experience and results on skeletal muscle changes in paraplegics, related to functional rehabilitation. Basic Appl Myol. 2001;11(2):75–85.
4. Rappl LM. Physiological changes in tissues denervated by spinal cord injury tissues and possible effects on wound healing. Int Wound J. 2008;5(3):435–44. [PMID:18205787] http://dx.doi.org/10.1111/j.1742-481X.2007.00360.x
5. Garber SL, Rintala DH, Hart KA, Fuhrer MJ. Pressure ulcer risk in spinal cord injury: Predictors of ulcer status over 3 years. Arch Phys Med Rehabil. 2000;81(4):465–71. [PMID:10768537] http://dx.doi.org/10.1053/mr.2000.3889

6. Chen Y, Devivo MJ, Jackson AB. Pressure ulcer prevalence in people with spinal cord injury: Age-period-duration effects. Arch Phys Med Rehabil. 2005;86(6):1208–13. [PMID:15954061] http://dx.doi.org/10.1016/j.apmr.2004.12.023
7. Rodriguez GP, Claus-Walker J. Biochemical changes in skin composition in spinal cord injury: A possible contribution to decubitus ulcers. Paraplegia. 1988;26(5):302–9. [PMID:3205571] http://dx.doi.org/10.1038/sc.1988.45
8. Polliack A, Taylor R, Bader D. Analysis of sweat during soft tissue breakdown following pressure ischaemia. J Rehabil Res Dev. 1993;30(2):250–59. [PMID:8035353]
9. Polliack A, Taylor R, Bader D. Sweat analysis following pressure ischaemia in a group of debilitated subjects. J Rehabil Res Dev. 1997;34(3):303–8. [PMID:9239623]
10. Sorichter S, Puschendorf B, Mair J. Skeletal muscle injury induced by eccentric muscle action: Muscle proteins as markers of muscle fiber injury. Exerc Immunol Rev. 1999; 5:5–21. [PMID:10519060]
11. Pepys MB, Hirschfield GM. C-reactive protein: A critical update. J Clin Invest. 2003;111(12):1805–12. [PMID:12813013]
12. Frost F, Roach MJ, Kushner I, Schreiber P. Inflammatory C-reactive protein and cytokine levels in asymptomatic people with chronic spinal cord injury. Arch Phys Med Rehabil. 2005;86(2):312–17. [PMID:15706560] http://dx.doi.org/10.1016/j.apmr.2004.02.009
13. Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. Am J Phys Med Rehabil. 2002;81(11 Suppl): S52–69. [PMID:12409811] http://dx.doi.org/10.1097/00002060-200211001-00007
14. Sorichter S, Mair J, Koller A, Pelsers MM, Puschendorf B, Glatz JF. Early assessment of exercise induced skeletal muscle injury using plasma fatty acid binding protein. Br J Sports Med. 1998;32(2):121–24. [PMID:9631217] http://dx.doi.org/10.1136/bjsm.32.2.121
15. Van Nieuwenhoven FA, Kleine AH, Wodzig WH, Hermens WT, Kragten HA, Maessen JG, Punt CD, Van Dieijen MP, Van der Vusse GJ, Glatz JF. Discrimination between myocardial and skeletal muscle injury by assessment of the plasma ratio of myoglobin over fatty acid-binding protein. Circulation. 1995;92(10):2848–54. [PMID:7586251]
16. Hagisawa S, Ferguson-Pell MW, Palmieri VR, Cochran GV. Pressure sores: A biochemical test for early detection of tissue damage. Arch Phys Med Rehabil. 1988;69(9): 668–71. [PMID:3421821]
17. Sari Y, Nakagami G, Kinoshita A, Huang L, Ueda K, Iizaka S, Sanada H, Sugama J. Changes in serum and exudate creatine phosphokinase concentrations as an indicator of deep tissue injury: A pilot study. Int Wound J. 2008;5(5): 674–80. [PMID:19134069] http://dx.doi.org/10.1111/j.1742-481X.2008.00543.x
18. Minematsu T, Nakagami G, Sari Y, Akase T, Sugama J, Nagase T, Sanada H. Candidate biomarkers for deep tissue damage from molecular biological and biochemical aspects. J Tissue Viability. 2010;19(2):77–83. [PMID:20223667] http://dx.doi.org/10.1016/j.jtv.2009.10.004
19. Scivoletto G, Fuoco U, Morganti B, Cosentino E, Molinari M. Pressure sores and blood and serum dysmetabolism in spinal cord injury patients. Spinal Cord. 2004;42(8):473–76. [PMID:15111999] http://dx.doi.org/10.1038/sj.sc.3101622
20. Morse LR, Stolzmann K, Nguyen HP, Jain NB, Zayac C, Gagnon DR, Tun CG, Garshick E. Association between mobility mode and C-reactive protein levels in men with chronic spinal cord injury. Arch Phys Med Rehabil. 2008; 89(4):726–31. [PMID:18374004] http://dx.doi.org/10.1016/j.apmr.2007.09.046
21. Marino RJ, Barros T, Biering-Sorensen F, Burns SP, Donovan WH, Graves DE, Haak M, Hudson LM, Priebe MM; ASIA Neurological Standards Committee 2002. International standards for neurological classification of spinal cord injury. J Spinal Cord Med. 2003;26(Suppl 1):S50–56. [PMID:16296564]
22. Clerico A, Del Chicca MG, Giampietro O, Fantoni M, Boldrini A. Factors affecting serum myoglobin levels in normal population. Ric Clin Lab. 1984;14(3):541–44. [PMID:6522965]
23. Pelsers MM, Chapelle JP, Knapen M, Vermeer C, Muijtjens AM, Hermens WT, Glatz JF. Influence of age and sex and day-to-day and within-day biological variation on plasma concentrations of fatty acid-binding protein and myoglobin in healthy subjects. Clin Chem. 1999;45(3):441–43. [PMID:10053065]
24. Wang TD, Wang YH, Huang TS, Su TC, Pan SL, Chen SY. Circulating levels of markers of inflammation and endothelial activation are increased in men with chronic spinal cord injury. J Formos Med Assoc. 2007;106(11):919–28. [PMID:18063513] http://dx.doi.org/10.1016/S0929-6646(08)60062-5
25. Segal JL, Gonzales E, Yousefi S, Jamshidipour L, Brunnemann SR. Circulating levels of IL-2R, ICAM-1, and IL-6 in spinal cord injuries. Arch Phys Med Rehabil. 1997; 78(1):44–47. [PMID:9014956] http://dx.doi.org/10.1016/S0003-9993(97)90008-3
26. Davies AL, Hayes KC, Dekaban GA. Clinical correlates of elevated serum concentrations of cytokines and autoantibodies in patients with spinal cord injury. Arch Phys Med Rehabil. 2007;88(11):1384–93. [PMID:17964877] http://dx.doi.org/10.1016/j.apmr.2007.08.004
27. Apple FS, Rogers MA, Sherman WM, Costill DL, Hagerman FC, Ivy JL. Profile of creatine kinase isoenzymes in

skeletal muscles of marathon runners. Clin Chem. 1984; 30(3):413–16. [PMID:6697488]
28. Evans WJ, Meredith CN, Cannon JG, Dinarello CA, Frontera WR, Hughes VA, Jones BH, Knuttgen HG. Metabolic changes following eccentric exercise in trained and untrained men. J Appl Physiol. 1986;61(5):1864–68. [PMID:3491061]
29. Adams JE 3rd, Abendschein DR, Jaffe AS. Biochemical markers of myocardial injury. Is MB creatine kinase the choice for the 1990s? Circulation. 1993;88(2):750–63. [PMID:8339435]
30. Glatz JF, Van der Vusse GJ, Simoons ML, Kragten JA, Van Dieijen-Visser MP, Hermens WT. Fatty acid-binding protein and the early detection of acute myocardial infarction. Clin Chim Acta. 1998;272(1):87–92. [PMID:9581860] http://dx.doi.org/10.1016/S0009-8981(97)00255-6
31. Miles MP, Andring JM, Pearson SD, Gordon LK, Kasper C, Depner CM, Kidd JR. Diurnal variation, response to eccentric exercise, and association of inflammatory mediators with muscle damage variables. J Appl Physiol. 2008; 104(2):451–58. [PMID:18079262] http://dx.doi.org/10.1152/japplphysiol.00572.2007
32. Rodriguez GP, Claus-Walker J. Measurement of hydroxylysine glucosides in urine and its application to spinal cord injury. J Chromatogr A. 1984;308:65–73. [PMID: 6746836] http://dx.doi.org/10.1016/S0021-9673(01)87533-6
33. Rodriguez GP, Garber SL. Prospective study of pressure ulcer risk in spinal cord injury patients. Paraplegia. 1994; 32(3):150–58. [PMID:8008417] http://dx.doi.org/10.1038/sc.1994.28
34. Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. Br Med Bull. 2007;81–82: 209–30. [PMID:17569697] http://dx.doi.org/10.1093/bmb/ldm014

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